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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 336814963 US

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☐ *Additional inventors are being named on the _____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

MODULATION OF CELL INTRINSIC STRAIN TO CONTROL MATRIX SYNTHESIS, SECRETION, ORGANIZATION AND REMODELING

19587 U.S. PTO
60/500049
09/04/03

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 5 ☐ CD(s), Number

☒ Drawing(s) *Number of Sheets* 6 ☒ Other (specify) Power Point Slides-39 sheets

☐ Application Data Sheet. See 37 CFR 1.76

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☒ Applicant claims small entity status. See 37 CFR 1.27.

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Respectfully submitted,

SIGNATURE Richard L. Byrne

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Date 09/04/03

REGISTRATION NO. *(if appropriate)* 28,498

Docket Number: 717-031727

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{W0078284.1}

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:	ATTORNEY'S DOCKET NUMBER
ALBERT J. BANES and JIE QI	717-031727

ENTITLED
MODULATION OF CELL INTRINSIC STRAIN TO CONTROL MATRIX SYNTHESIS, SECRETION, ORGANIZATION AND REMODELING

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SPECIFICATION (5 pp.); SIX (6) SHEETS OF FIGURES;
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MODULATION OF CELL INTRINSIC STRAIN TO CONTROL MATRIX SYNTHESIS, SECRETION, ORGANIZATION AND REMODELING

[0001] Cells fabricate, organize and strengthen a matrix by a mechanism described as "structural tensioning" driven by "tractional structuring". Cells maintain their own setpoint for a basal intrinsic strain level determined in part by their connectivity to the substratum, internal architecture that balances the external and internal forces and their propensity to move along the matrix and organize it. Cells respond to extrinsic tension by adjusting their shape, connections to matrix and other cells and internal tension. Thus cells develop an "intrinsic strain" for a given extrinsic strain and attempt to modulate their cell-matrix contacts, pseudopod lengths, degree and types of cytoskeletal organization and modulus of elasticity based on the intrinsic strain value.

[0002] One can manipulate the intrinsic strain in cells and a forming tissue by modulating the cell's connections to its matrix or by modulating the internal strain (actual or perceived) with or without the synergistic or antagonistic action of applied mechanical loading. This modulation is regulated in the cell through connections to the matrix by matrix attachment proteins such as integrins, connections through cytoskeletal filaments or by pathways that modulate the cell-matrix connections and/or cytoskeleton at the plasma membrane at the endoplasmic reticulum and at the nucleus. One can treat a tissue engineered construct with mediators that cause a release of cell attachment points to the matrix such as peptides that compete for the attachment sites. One such peptide is the collagen, elastin or fibronectin binding site peptide involving the arginine-glycine-aspartic acid sequence (-RGD-). Another peptide is the laminin binding peptide comprised of tyrosine, isoleucine, glycine, serine and arginine (-YIGSR-). Other peptides or mediators might modulate attachment to proteoglycans such as decorin, biglycan, fibromodulin, lumican or others. In this way, one can regulate the cell's shape and its synthetic expression phenotype. Treatment of cells in a matrix or about to be seeded in a matrix will then modulate attachment to the matrix and subsequently moderate their tensional structuring of the matrix by tractional structuring. One can also modulate attachment and tensional structuring by adding ligands such as ATP or UTP (adenosine triphosphate or uridine triphosphate) which cause a relaxation of the cells through a purinoceptor-driven pathway (P2Y or P2X). A corollary of this strategy is that the cells may express more matrix or more of a given matrix component such as a collagen or elastin or proteoglycan or become more highly cross-linked in response to a change in intrinsic strain. In this way, the matrix becomes more organized, with a more native matrix phenotype and will be stronger to resist applied strain. One can also add matrix components

to the tissue engineered construct at the beginning, during or at the end of fabrication and culture to modulate attachment to the matrix. A component such as hyaluronic acid can reduce matrix remodeling, for instance.

[0003] Another method one can use to modulate the intrinsic strain that the cell senses is to adjust the internal strain of the cell with a mediator +/- mechanical strain. A cytokine such as interleukin 1 beta (IL-1b) can be given to the cell to act in at least two ways: 1) to modulate expression of cytoskeletal genes and synthesis of cytoskeletal proteins such as actin, myosin, α-actinin, vimentin, vinculin, titin and others, and 2) to modulate expression of matrix metalloproteinases (MMPs) which when activated, can degrade the matrix. Other mediators such as cytochalasin b, can interfere with actin polymerization and thus decrease the modulus of the cell and its internal strain. Potentially, one can also use RNA silencing techniques of other gene expression modulating techniques to reduce expression of any of these or other genes which would impact the internal strain setpoint of the cell. The strategy is that once one resets the internal strain setpoint of the cell by altering the cytoskeleton profile, ratio and structure, the cells will respond by making more, resetting their remodeling regimen and make an even more organized and robust matrix with greater mechanical strength.

[0004] Particularly, one can modulate the cell in this way to direct matrix remodeling through matrix organization, degradation and/or matrix synthesis.

[0005] The end result may be increased matrix build-up and/or organization or reorganization, yielding a tissue engineered construct with greater strength to endure the rigors of the native biomechanical environment. This process can occur via manipulation of connections to the matrix externally, thus affecting the strain setting of the cell, or by manipulating the internal architecture of the cell, thus affecting the strain setting, or both affects can be used simultaneously or sequentially.

[0006] Cells maintain an intrinsic setpoint for strain mediated by attachment to the matrix as well as arrangement of cytoskeletal filament proteins. In tissues, these attachments to collagens and/or proteoglycans impart to the cell a given shape with either extensive cell processes as in many connective tissue cells such as those in tendon or ligament or bone, or few processes as in chondrocytes at weight bearing cartilage.

[0007] One can culture cells in a matrix material (s) in a bioreactor either outside the body or within the body for the purposes of engineering a tissue to replace, augment or repair a damaged native tissue or provide a missing tissue. One drawback to this method is that cells produce a matrix of limited strength without a defined matrix phenotype. This lack of

definition to the matrix phenotype usually results in a weak tissue that cannot withstand the rigors of the native biomechanical environment. We believe that cells in engineered tissues or even in natural tissue can be modulated by chemical ligands to alter their intrinsic strain environment such that the cells remodel the surrounding matrix and make it stronger and more organized. In particular, ligands such as cytokines of the interleukin category (IL-1b) can be used to modulate both the matrix metalloproteoinase expression pattern in cells as well as the cytoskeleton pattern. This modulation can favorably affect the strength and arrangement of the cytoskeleton inside the cell as well as the matrix outside the cell. Other mediators such as ATP or UTP can be used to modulate the expression patterns further to reduce expression of the MMPs. In addition, adding particular regimens of mechanical loading of the constructs can synergize with the effects of the ligands in common and/or intersecting pathways that further modulate the effects of the ligands and result in cells that can withstand mechanical loading. Doses of ligands and mechanical loading can be used that accentuate expression of collagens and elastins as well as particular cytoskeletal filaments. The alteration in cytoskeleton filament profiles can modulate the cell stiffness resulting in a cell that can better resist externally applied or internally applied loads. These mechanisms can be used to manipulate the cell's expression patterns for both matrix, cell attachment proteins, cytoskeletal binding partners, pathway modulators and cytoskeletal proteins to yield cells and matrices that are stonger than nontreated counterparts and can better withstand the rigors of the biomechanical environment.

CLAIMS:

1. Cells develop an "intrinsic strain" for a given extrinsic strain and attempt to modulate their cell-matrix contacts, pseudopod lengths, degree and types of cytoskeletal organization and modulus of elasticity based on the intrinsic strain value.

2. One can manipulate the intrinsic strain in cells and a forming tissue by modulating the cell's connections to its matrix or by modulating the internal strain (actual or perceived) with or without the synergistic or antagonistic action of applied mechanical loading.

3. One can treat a tissue engineered construct with mediators that cause a release of cell attachment points to the matrix such as peptides that compete for the attachment sites.

4. Treatment of cells in a matrix or about to be seeded in a matrix will then modulate attachment to the matrix and subsequently moderate their tensional structuring of the matrix by tractional structuring.

5. One can also modulate attachment and tensional structuring by adding ligands such as ATP or UTP (adenosine triphosphate or uridine triphosphate) which cause a relaxation of the cells through a purinoceptor-driven pathway (P2Y or P2X).

6. A correlary of this strategy is that the cells may express more matrix or more of a given matrix component such as a collagen or elastin or proteoglycan or become more highly cross-linked in response to a change in intrinsic strain.

7. The matrix becomes more organized, with a more native matrix phenotype and will be stronger to resist applied strain. One can also add matrix components to the tissue engineered construct at the beginning, during or at the end of fabrication and culture to modulate attachment to the matrix. A component such as hyaluronic acid can reduce matrix remodeling, for instance.

8. One can use to modulate the intrinsic strain that the cell senses is to adjust the internal strain of the cell with a mediator +/- mechanical strain. A cytokine such as interleukin 1 beta (IL-1b) can be given to the cell to act in at least two ways:

a. to modulate expression of cytoskeletal genes and synthesis of cytoskeletal proteins such as actin, myosin, α-actinin, vimentin, vinculin, titin and others, and

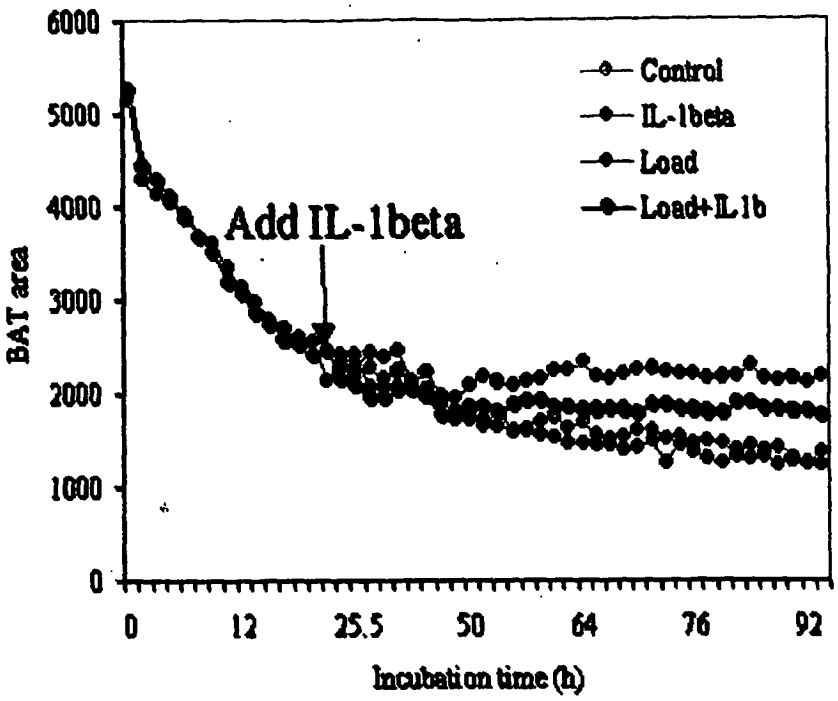
b. to modulate expression of matrix metalloproteinases (MMPs) which when activated, can degrade the matrix.

9. Mediators such as cytochalasin b, can interfere with actin polymerization and thus decrease the modulus of the cell and its internal strain.

10. One can also use RNA silencing techniques of other gene expression modulating techniques, to reduce expression of any of these or other genes which would impact the internal strain setpoint of the cell. The strategy is that once one resets the internal strain setpoint of the cell by altering the cytoskeleton profile, ratio and structure, the cells will respond by making more resetting their remodeling regimen and make an even more organized and robust matrix with greater mechanical strength.

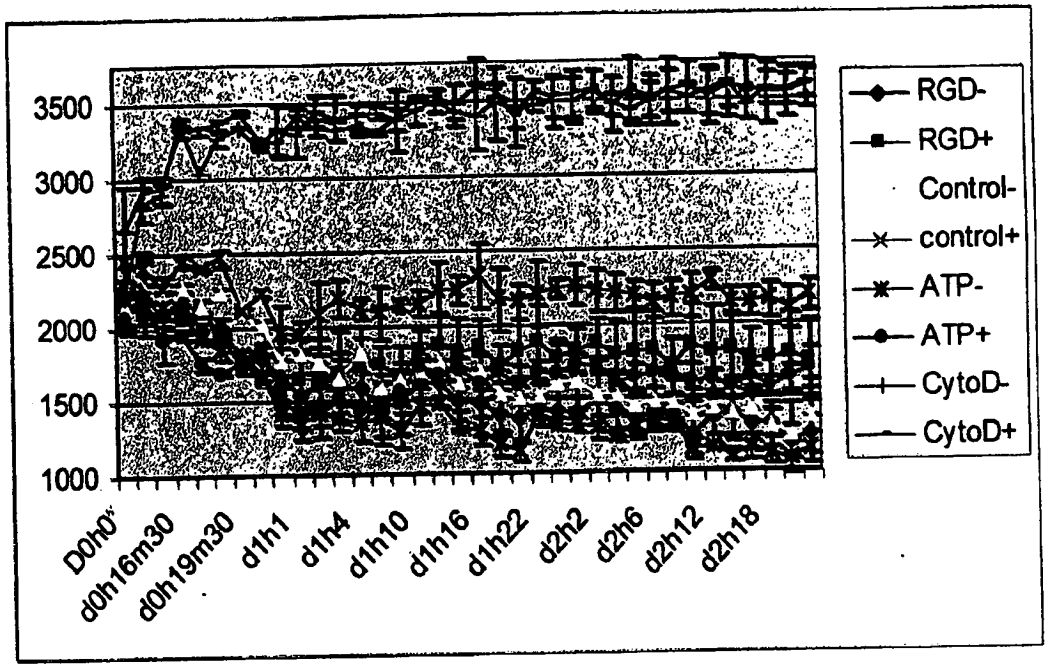
11. Particularly, one can modulate the cell in this way to direct matrix remodeling through matrix organization, degradation and/or matrix synthesis.

12. The end result may be increased matrix build-up and/or organization or reorganization yielding a tissue engineered construct with greater strength to endure the rigors of the native biomechanical environment. This process can occur via manipulation of connections to matrix externally thus affecting the strain setting of the cell or by manipulating the internal architecture of the cell thus affecting the strain setting, or both affects can be used simultaneously or sequentially.



Data in Figure 1 indicate that the rate of matrix remodeling can be regulated by mediators that affect both the synthesis of matrix metalloproteinases that release cells from matrix as well as cytoskeleton proteins that control the internal architecture of the cell to resist outside forces as well as those generated internally. Cells embedded in a linear, tethered bioartificial matrix contract or remodel their surrounding biomaterial (in this case, collagen fibrils) in a rate-dependent manner (light blue dotted line). Cells attach to the matrix via integrin contacts then extend pseudopods and engulf the collagen fibrils. Certain mechanical loading regimens can reduce (black dotted line) or modulate the contraction/remodeling rate of the matrix (dark blue dotted line). Moreover, the cytokine IL-1b, can reduce the contraction/remodeling rate of the ells for the matrix (red dotted line). Cells then can be modulated with mediators to control the rate of remodeling of their matrix.

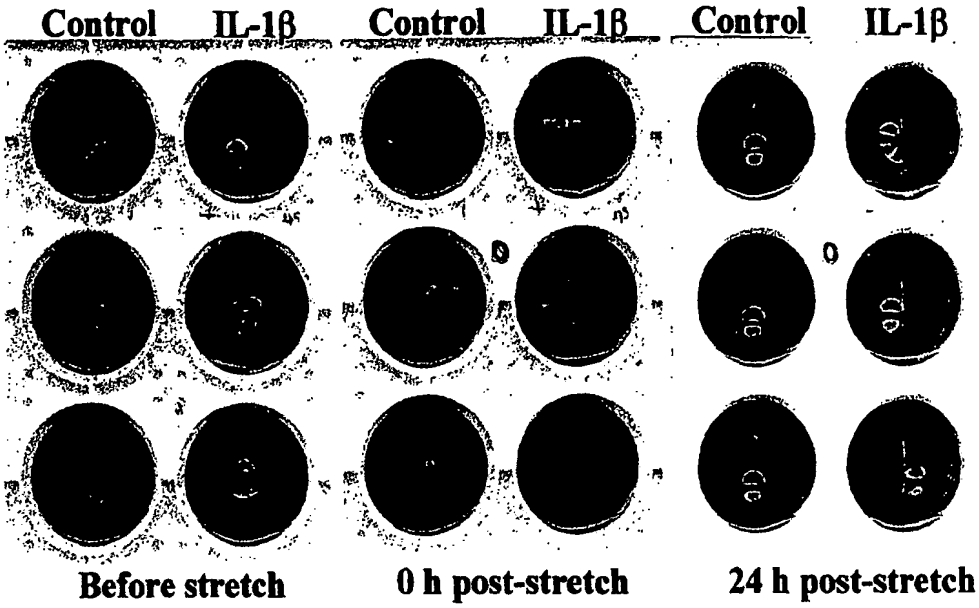
Figure 1



Data in Figure 2 indicate that the matrix remodeling rate of bioartificial tissues can be modulated by ligands and mechanical load. Compounds that interact at either the cell attachments to matrix (RGD, dark blue and light pink), ligands that affect polymerization of internal structural, cytoskeleton components (cytochalasin D, green and light blue) or ATP that interacts at purinoceptors (brown or red), can alter the remodeling rate of tissue engineered matrices. In this case, cytochalasin D which prevents polymerization of G to F-actin was most effective in preventing matrix contraction/remodeling.

Figure 2

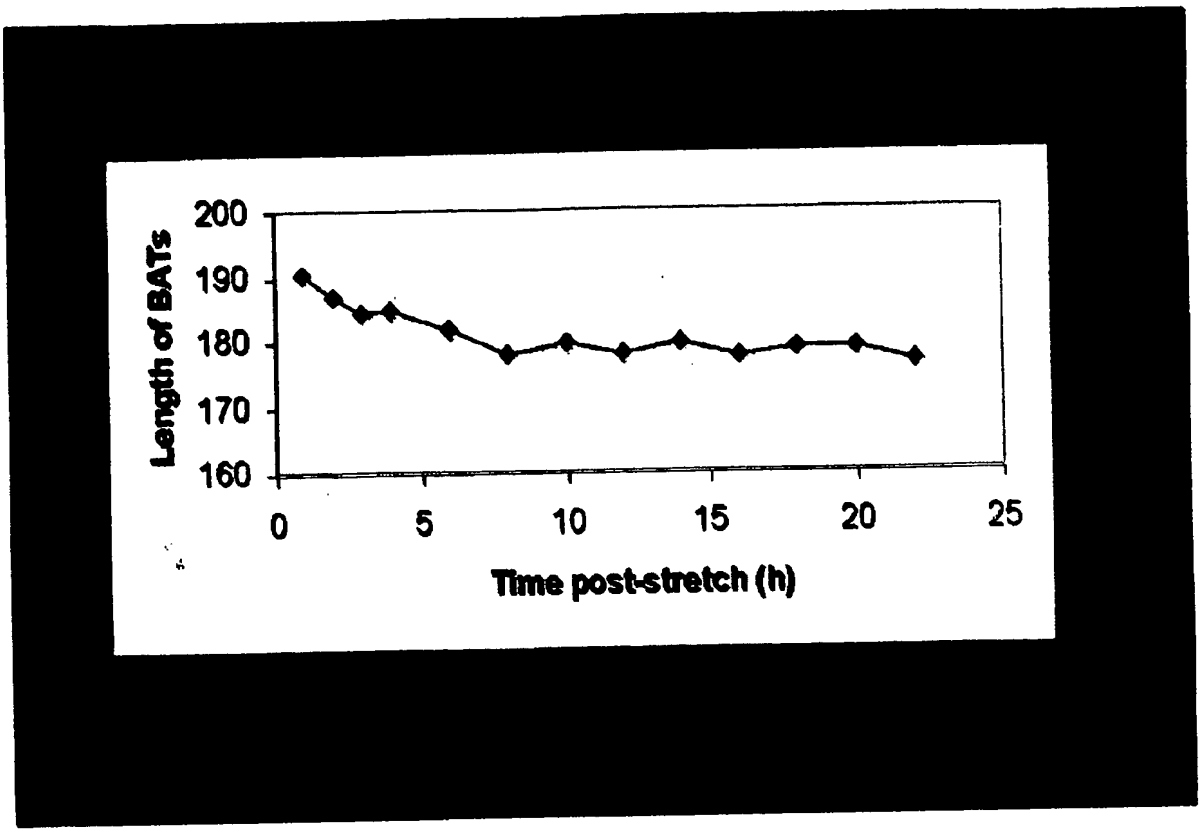
hBATs: IL-1β increases Elastic Recovery



Loading condition: 15% elongation, 1 Hz, 1 h

Pictures in Figure 3 illustrate the thought that the cytokine, IL-1b, protects bioartificial tissues (BATs), from the consequences of excessive mechanical loading (15% elongation, 1 Hz, 1h). Tendon cells were plated in TissueTrainTM culture plates in linear, tethered constructs at 200k cells/200 microliters collagen gel. Cells were incubated for 3 days then pre-treated with 1 nM IL-1b or not, then subjected to excessive mechanical load at 15% elongation at 1 Hz for 1 h. Cells that were pretreated with IL-1b and were exposed to IL-1b throughout the course of loading and for the period thereafter were protected from breakage (extension to failure). IL-1b-treated BATs sustained elastic deformation and were stretched beyond the original limits of the BAT. BATs that did not receive IL-1b failed at their midpoint.

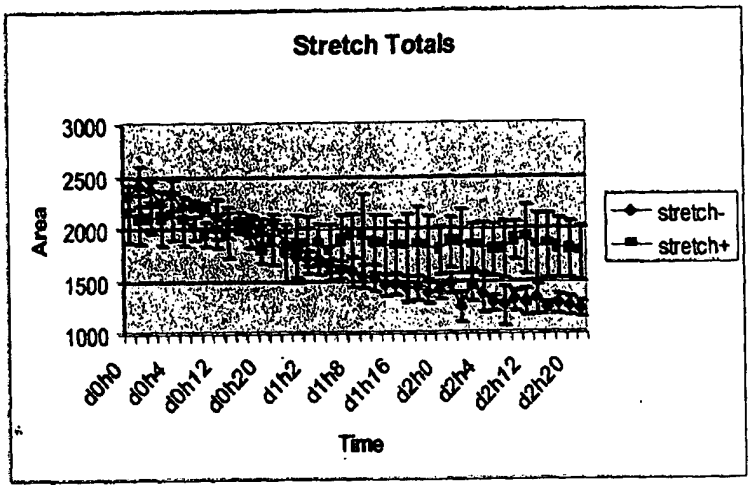
Figure 3



Data in Figure 4 indicate that cells in IL-1b-treated BATs that were subjected to 15% elongation at 1Hz for 1 h survive the strain regimen intact, are elongated by the mechanical loading, but recover their original length by approximately 8 hours. Cells sustain this length for an additional 16 h.

Figure 4

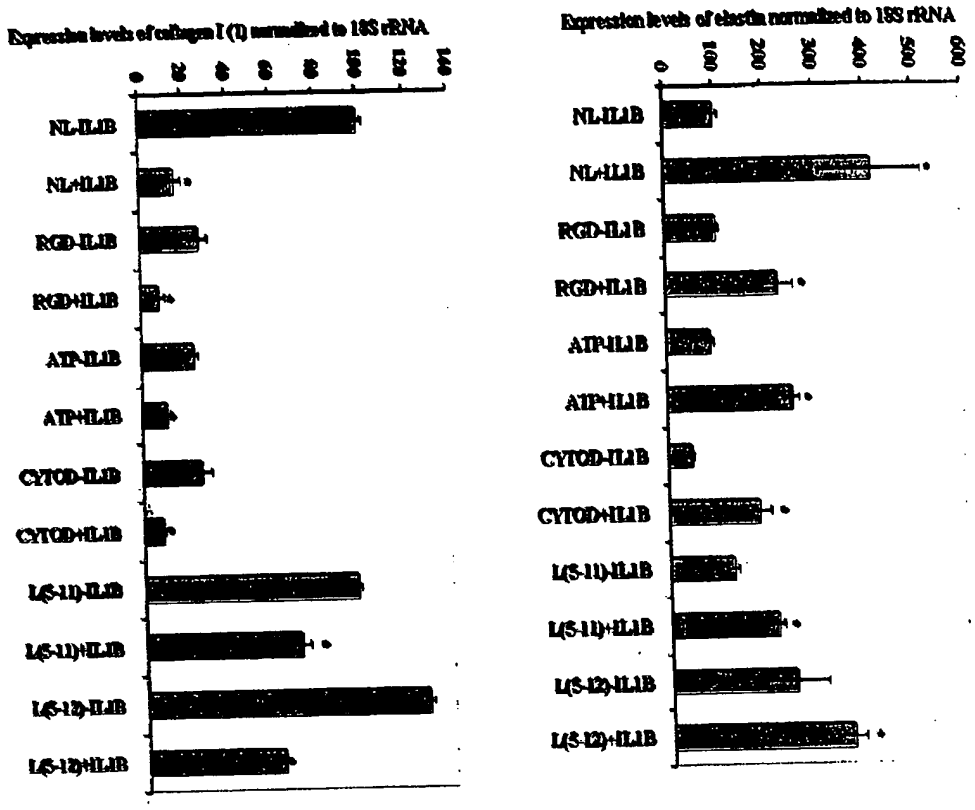
hBATs: IL-1b and Stretch Retard 3D Gel Contraction/Remodeling



Data in Figure 5 indicate that cells in BATs treated with IL-1b (pink line) from the onset of the experiment show delayed or retarded matrix contraction throughout the course of a 3 day experiment where matrix contraction is most dynamic (blue line).

Figure 5

3D BATs: Collagen Gene is "off" but Elastin is "on" by IL-1 β



Data in Figure 6 indicate that the collagen type I gene is repressed by IL-1b(NL+ IL-1b) but that for elastin is stimulated (NL+ IL-1b). Modulation of cell shape by attachment to the matrix or through the cytoskeleton or related pathways repressed collagen synthesis during this time frame. Elastin synthesis in groups that received modulators and IL-1b was stimulated, particularly with load.

Figure 6

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